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**APPLICATION FOR
UNITED STATES LETTERS PATENT**

Applicant: Steven T. Boyce

Title: APPARATUS FOR PREPARING A
BIOCOMPATIBLE MATRIX

ASSIGNEES: University of Cincinnati
-and-
Shriners Hospitals for Children

Beverly A. Lyman
Wood, Herron & Evans, L.L.P.
2700 Carew Tower
Cincinnati, OH 45202-2917
Attorneys
(513) 241-2324 telephone
(513) 421-7269 facsimile

Attorney Reference No.: CUT-02

SPECIFICATION

APPARATUS FOR PREPARING A BIOCOMPATIBLE MATRIX

Field of the Invention

The invention is directed generally to an apparatus and method to prepare a biocompatible reticulated matrix that may be used to support cells to form a surgical device such as a cultured skin substitute device.

5 Background

Skin is one of the largest organs in the body and covers substantially the entire body surface. Skin is composed of two main layers: the surface epithelium or epidermis, which contains keratinocytes as one type of epidermal cells, and the subjacent connective tissue layer or dermis, which 10 contains fibroblasts as one type of dermal cells. The functions of skin include protecting an organism from injury and dessication by serving as a barrier to infection, perceiving or detecting environmental stimuli, excreting various substances, regulating body temperature, and helping to maintain water balance. Because of its quantitative and qualitative importance, substantially 15 intact and healthy skin is crucial, not only for the well being of an organism but for its very survival.

The health and integrity of skin may be compromised by congenital or acquired pathological conditions, either acute or chronic, for which normal skin regeneration and repair processes may be inadequate. These conditions include, but are not limited to, burns, wounds, ulcers, infections, 5 and/or congenital abnormalities. Patients who are burned over a large surface area often require immediate and extensive skin replacement. Less life-threatening but chronic skin conditions, as occur in venous stasis ulcers, diabetic ulcers, or decubitus ulcers as three examples, may progress to more severe conditions if left untreated, particularly since patients with these 10 conditions have an underlying pathology. Reduction of morbidity and mortality in such patients depends upon timely and effective restoration of the structure and function of skin.

Skin substitutes derived either *ex vivo* or *in vitro* may be used to treat these or other conditions. Desirable skin substitutes are readily available, 15 require a minimum amount of donor skin, are otherwise simple to produce, and are cost-effective to generate and maintain. Several approaches to produce skin substitutes which satisfy some or all of these requirements have been attempted, with varying degrees of success. However, no skin substitute has yet regenerated all of the structures and functions of skin. Rather, all are 20 subsets of uninjured skin. Only a transplant of full thickness skin, which demonstrates scarring during healing, and restores virtually all structures and functions of normal uninjured skin.

Materials have been manufactured for use in permanent skin repair. These materials contain different components replacing or simulating 25 the components and functions of the dermis and/or epidermis. Examples of

these materials include EpiCel™, which lacks a dermal component and uses the patient's own cultured keratinocytes; Integra™, which uses a collagen-glycosaminoglycan (GAG) matrix to provide an acellular dermal component and uses a thin epidermal autograft; AlloDerm™, which uses a dermal matrix and a thin epidermal autograft; DermaGraft™, which uses a polyglycolic acid/polylactic acid (PGA/PLA) matrix and allogeneic human fibroblasts for the dermis; Hyaff/LaserSkin™, which uses hyaluran and fibroblasts for the dermis, and hyaluran and the patient's own keratinocytes for the epidermis; and PolyActive™, which uses polyethylene oxide/polybutylthalate (PEO/PBT) and the patient's own fibroblasts for the dermis, and the patient's cultured keratinocytes for the epidermis.

Materials to either temporarily cover wounds, or to stimulate permanent skin repair processes, included ApliGraft™, which uses collagen gel and allogeneic fibroblasts for the dermis, and cultured allogeneic keratinocytes for the epidermis; Comp Cult Skin™ or OrCel™, which uses collagen and allogeneic fibroblasts for the dermis, and cultured allogeneic keratinocytes for the epidermis; and TransCyte™, which uses allogeneic fibroblasts for the dermis and a synthetic material, BioBrane™, for the epidermis.

While the above materials are useful to varying degrees, each has disadvantages and limitations. Some of the materials are mechanically fragile, making it difficult to perform the required manipulations and transfers of the material in large sections without tearing. Instead, the materials must be used as smaller pieces, which makes coverage of large surface areas technically laborious for the physician and cosmetically undesirable for the patient. The materials are also susceptible to microbial contamination, which is

unacceptable for patients who are already at an increased risk for infection due to their compromised condition. The materials show varying rates of engraftment and time to heal, both of which must be considered in balancing the advantage of a particular material over another for a particular patient. For example, a material which is otherwise acceptable but which takes longer to engraft and heal is less desirable, since recovery includes as rapid a return to a normal routine as possible.

Yannas et al. in U.S. Pat. No. 4,458,678 discloses a method for preparing a fibrous lattice and seeding it with viable cells. The lattice is prepared by pouring an aqueous slurry of collagen and glycosaminoglycan into an open metal tray or pan. Use of an open tray results in asymmetric contact of the slurry to ambient conditions; that is, one surface of the slurry is in contact with a surface of the tray or pan ("pan surface"), while another surface of the slurry is in contact with the atmospheric environment ("air surface"). The pan is then placed in contact with a heat sink, such as the refrigerated shelf of a lyophilizer, to initiate freezing of the aqueous slurry from the pan side. Freezing proceeds predominantly in a vectorial direction from the pan surface to the air surface, with heat flowing much more rapidly toward the pan surface than the air surface. Upon freezing, the structure of the lattice is asymmetric with, generally, more frequently distributed reticulations of polymers toward the pan surface than the air surface. After freeze-drying, the resulting lattice is an asymmetric structure, which results in non-uniform ability for cells to penetrate the lattice. Furthermore, the method and apparatus of Yannas limit both the uniformity of thickness, and the minimum thickness that can be obtained consistently and deliberately.

The inventor's own previous composite skin replacement, disclosed in U.S. Patent No. 5,976,878, which is expressly incorporated by reference herein in its entirety, has been used successfully for permanent skin replacement. It is applied surgically in a single procedure, and contains a layer

5 of cultured epidermal cells, a synthetic dermal membrane component, and a substantially nonporous synthetic lamination layer on one surface of the dermal membrane component. The synthetic dermal membrane component is formed from collagen, or collagen and a mucopolysaccharide compound, and is laminated with the same collagen or collagen and mucopolysaccharide

10 compound-containing solution containing a volatile cryoprotectant. The substantially nonporous lamination layer may be located between the dermal component and the layer of cultured epidermal cells, promoting localization of epidermal cells on the surface of the dermal component and movement of nutrients to the cells of the cellular epidermal component. This composition

15 can also be used to deliver biologically active molecules to the site where it is applied. The apparatus for preparing the synthetic dermal membrane component, used to prepare the composite skin replacement of the '878 patent, is disclosed in U.S. Patent No. 5,711,172, which is expressly incorporated herein by reference in its entirety.

20 Desirable features of the above-described composite skin replacement included an increased rate of vascularization of the area covered by the material, decreased microbial contamination, increased nutrient supply, and improved epidermal barrier function, compared to other materials. Areas covered with the composite skin replacement required less time to engraft and

25 heal, and the material was less susceptible to microbial contamination

compared to other materials. Other desirable features were that this material was relatively non-fragile and easy to handle, and could be generated relatively rapidly, for example, within the time frame in which a burn patient would require skin grafts. However, while no other alternative material has healed excised, 5 full thickness wounds more rapidly, and with as low an incidence of microbial contamination, limitations still exist. Thus, there remains a need to more closely approach the structural and functional properties of normal uninjured skin.

Summary of the Invention

10 The invention is directed to an apparatus for the preparation of a biocompatible, biodegradable, matrix having substantially symmetrical reticulations to support cells inoculated thereon. The cells may be cultured skin cells for formation of an engraftable device for the surgical replacement of skin in burn patients, for example. Basically, a solution of matrix components is cast 15 between top and bottom surfaces (e.g., plates) of a material which facilitates rapid transfer of heat, so that the solution in contact with these surfaces is rapidly and uniformly cooled on both top and bottom surfaces. Once frozen, the solid matrix has substantially symmetric reticulations, which allow uniform cell population and result in an improved device for ingrafting or implanting in a 20 patient. The apparatus and method of using the apparatus results in reproducible matrices each time the apparatus is used, so that reproducible devices are generated.

 In one embodiment, the apparatus contains a chamber defined by top and bottom planar surfaces of a heat conductive material, such as a metal. 25 The sides of the chamber are defined by at least one gasket of substantially

uniform thickness positioned between the top and bottom surfaces. Closures, such as clips, latches, clamps, etc. hold the planar surfaces together. The assembled apparatus allows indirect contact by a coolant and subsequent freezing of the matrix-forming solution. The chamber may be immersed in the

5 coolant, either vertically or horizontally, as in a coolant bath.

In another embodiment, the apparatus containing the above-described chamber may be further contained within a jacket through which a coolant flows or recirculates. In this embodiment, the apparatus also has top and bottom frames which are affixed to the top and bottom surfaces of the heat

10 conductive material. The top and bottom frames contain one or more ports and channels through which a coolant fluid flows. The embodiment provides a contained apparatus to effect the desired rate of cooling.

In another embodiment, the invention is directed to a method of preparing the matrix using the apparatus. A matrix-forming solution is

15 contained within a chamber and is in contact with a material which allows rapid and substantially uniform removal of heat from the top and bottom surfaces of the solution. Gaskets of a desired thickness separate the top and bottom surfaces and allow control over the thickness of the resulting matrix. Using a coolant that can effect the desired rate of cooling, the solution contained in the

20 chamber is frozen to form a solid matrix. The solid matrix is then dehydrated and crosslinked using physical methods such as freeze-drying and thermal crosslinking, rather than by adding chemical crosslinking compounds, the residue of which may negatively affect the formed matrix and the cells with which it comes in contact. The resulting physically-crosslinked matrix is then

25 sterilized before cells are inoculated. The matrix thus prepared has a thickness

and reticulation pattern which facilitates uniform population with cells and results in a device which is readily engrafted or transplanted into a patient.

These and other features of the invention will be further appreciated with reference to the following drawings and detailed description.

5 **Brief Description of the Drawings**

FIG. 1A shows components of one embodiment of a matrix-casting apparatus.

FIG. 1B shows the assembled apparatus.

FIG. 1C is a cross-section view of the apparatus.

10 FIG. 1D shows the components of a second embodiment of the matrix-casting apparatus.

FIG. 1E is a cross-section view of the second embodiment of the matrix-casting apparatus

FIG. 2A is a top view of the bottom frame of the apparatus.

15 FIG. 2B is a bottom view of the bottom frame of the apparatus.

FIG. 2C is a side view of the bottom frame of the apparatus.

FIG. 3A is a bottom view of the center frame of the apparatus.

FIG. 3B is a side view of the center frame of the apparatus.

20 FIG. 3C is a cross section view of the center frame of the apparatus.

FIG. 4A is a top view of the top frame of the apparatus.

FIG. 4B is a side view of the top frame of the apparatus.

FIG. 4C is a cross section view of the top frame of the apparatus.

Detailed Description

The invention is a method and apparatus to prepare an acellular biocompatible biodegradable matrix having substantially uniform and symmetric reticulations that are formed by freezing an aqueous fluid containing protein, or 5 protein and carbohydrate, at a controlled rate, and subsequent freeze-drying (lyophilization) and a uniform thickness of less than 1 mm. The reticulated matrix is capable of serving as a support or scaffold for cells inoculated thereon. The cells are animal cells, which encompass human cells. In one embodiment, the cells are epidermal and dermal cells, resulting in a cultured 10 skin device as disclosed in co-pending related U.S. Patent Application Serial No. _____, entitled Surgical Device For Skin Therapy or Testing and filed on even date herewith, which is expressly incorporated by reference herein in its entirety. The device prepared using the inventive apparatus facilitates cell attachment to the multiple continuous surfaces of the matrix and maintains a 15 desired thickness. This is in contrast to a matrix with asymmetric reticulations and less uniform thickness that is formed when a fluid containing protein, or protein and carbohydrate, contacts an open tray or pan during matrix formation.

Biological incorporation of a matrix into or overlying a wound depends, in part, upon the ability of non-inflammatory connective tissue cells to 20 migrate into the matrix from the wound bed. Thus, the structural and biochemical characteristics of the matrix must be uniform, and further must be optimized to promote fibrovascular migration. The inventive method and apparatus controls structural characteristics of the matrix, such as its thickness and the uniformity and size of the resulting reticulations, by the symmetric 25 extraction of heat from the matrix-forming fluid and the rigid and co-planar

surfaces from which heat is extracted. This results in a matrix with symmetric reticulations and uniform thickness. Other variables that are controlled include the rate of cooling to effect freezing the matrix-containing fluid, the size of the ice crystals formed, the volume of the matrix-forming fluid that is frozen, and the
5 concentration (% wt/vol) of the components in the matrix-forming fluid.

In one embodiment, the invention is an apparatus to prepare a biocompatible degradable reticulated matrix. The apparatus has a closed chamber to contain a matrix-forming fluid, such as a protein containing fluid. The chamber may be defined by six sides, as in a box. In this embodiment, two
10 of the sides are heat conductive (e.g., metal), parallel rectangular plates that are made more rigid by a framework of reinforcing supports outside of the chamber that contains the matrix-forming fluid. The remaining four sides of the chamber may consist of material that acts as a gasket or O-ring, and may be adjusted in thickness to define the thickness of the resulting matrix. In one
15 embodiment, the gasket consists of two pieces of material. One piece has two parallel strips connected at one end by a perpendicular strip (U-shaped) that are located to adjoin three of the four edges of the two plates. The matrix-forming fluid may be introduced into the chamber through the side without a gasket (open side). Subsequently, the second gasket piece is placed between
20 the fourth edges of the two parallel plates as a single strip to contain the matrix-forming solution. In another embodiment, the gasket is a singular, rectangular strip or O-ring adjoining the perimeters of the two parallel plates, and may have ports for introduction of the matrix-forming solution and displacement of air.

The thickness of the matrix may be adjusted by the thickness of
25 the gasket material used in the chamber. The shape of the chamber need not

be rectangular, nor the resulting matrix planar. The chamber may also be a continuous surface of any shape, as in a bladder, sack, or other type of flexible container that may be selectively deformed to obtain the desired shape and thickness of the matrix with ports for introduction of matrix-forming solution and
5 displacement of air. The surface of the chamber is also removable to expose a surface for freeze-drying.

The matrix-forming fluid contained in the chamber is frozen by the removal of heat at a controlled rate. This occurs predominantly through the two plates by simultaneous and symmetric application of a coolant to the opposite
10 sides of the plates. Application of the coolant may be accomplished by several mechanisms. As one example, the sealed chamber containing the matrix-forming solution may be submerged or flooded with a chilled liquid coolant in an open bath of a static coolant. As another example, a coolant may be contained in a closed, recirculating system that flows in contact with the parallel plates of
15 the chamber. As yet another example, a liquid or gaseous coolant may flow through an open vessel and be exhausted without recirculation. To establish a closed, recirculating coolant system, the plates between which the matrix-forming solution is placed and sealed may themselves be incorporated into a single apparatus, such as two containers through which the coolant flows
20 (coolers). In one embodiment, the plates contacting the matrix-forming solution comprise one surface each of two hollow boxes with ports for supply and return vessels (i.e., tubing) of the coolant to a recirculating chiller, or for supply and exhaust of a flowing, non-recirculating coolant, such as liquid nitrogen.

The structure of any given formulation of matrix may then be
25 regulated by controlling the rate of freezing. This may be accomplished in a

number of ways, for example, by adjustment of the coolant temperature and volume in an open system; by adjustment of the coolant temperature and flow rate in a closed system; by selection of a coolant having a specific heat capacity; or by selection of the materials for the chamber and/or apparatus with preferred conductivities of heat. The structure of the matrix may also be regulated by the formulation of the matrix-forming solution.

The frozen matrix is subsequently dehydrated, crosslinked with or without the use of chemical crosslinking agents, and sterilized to provide a support or scaffold on which cells may be inoculated. The controlled and symmetric rate of freezing of the matrix produces uniform and symmetric reticulations throughout the uniform thickness of the matrix, which facilitate uniform cell distributions in the matrix and provide an enhanced surgical device.

In one embodiment, as shown in FIGS. 1A-B, the matrix-forming apparatus **30** consists of three frames that, upon assembly, form a preparation device or freezing apparatus. The three frames are a bottom frame **10**, a center frame **15** which is also referred to as a carrier plate, and a top frame **25**. Frames **10**, **15**, and **25** may be constructed of a metal, such as aluminum or steel, or another inflexible material such as wood, ceramic, or hard plastic. Apparatus **30** also includes a resilient spacer gasket **20**, a spacer gasket plug **21**, and "O" ring gaskets (not shown) which may be silicone rubber or other synthetic material.

Center frame **15** has a top surface **51b**, and top frame **25** has a bottom surface **51c**, both surfaces **51b**, **51c** may be of any non-adherent material that resists deformation (for example, polished aluminum or other metal, ceramic, etc.) to facilitate removal of the subsequently formed

matrix. Alternatively, a thin sheet **53** of a non-stick material such as TEFLON™ may be placed between spacer gasket **20** and top frame **25** to provide an additional non-adherent surface to facilitate separation of bottom surface **51c** from the frozen matrix. The surfaces **51b**, **51c** may also be coated with a non-adherent material. Spacer gasket **20** and spacer gasket plug **21** may be made of a silicone rubber or other appropriate material.

With reference to FIGS. 1D and 1E in which the reference numerals refer to like features in FIGS. 1A - 1C the matrix-forming apparatus **30a** may optionally be used independent of the bottom frame **10** and top plate **50d** of apparatus **30**. This embodiment is useful for exposure of the apparatus **30** containing the matrix-forming solution to a static coolant. This embodiment also includes a top frame **25a** without sheet **50d** so that the coolant may be in direct contact with surface **51d** of sheet **50c**, a center frame **15a**, also called a carrier plate, having a fastener such as a one half draw latch **45** adapted to the outside surfaces of the frame walls **61a-d**, a spacer gasket **20**, a spacer gasket plug **21**, and optionally a non-stick sheet **53**.

The frames **10**, **15**, **25** and gaskets **20**, **21** are assembled to form the apparatus **30** by fastening the components together as shown in FIG. 1B. As shown in FIG. 1C within the assembled apparatus **30** is a chamber **75** capable of containing the matrix-forming fluid for subsequent controlled-rate freezing. The chamber **75** is defined by the following surfaces: the inner walls **22a-c** of the spacer gasket **20**, the inner wall **23** of the spacer gasket plug **21**, the top surface **51b** of the center frame **15** with or without the optional non-stick sheet **53**, and the bottom surface **51c** of the top frame **25**. The spacer gasket **20** and spacer gasket plug **21** are of the same thickness, which regulates the

thickness of the resulting matrix formed within the apparatus 30. In one embodiment, the gasket 20 and gasket plug 21 have a thickness in the range of about 0.1 mm to about 10 mm. In another embodiment, the gasket 20 and gasket plug 21 have a thickness in the range of about 0.5 mm to about 1.5 mm.

- 5 Each of these components of the apparatus 30 will now be described in further detail.

Bottom Frame

The components of the bottom frame 10 are shown in FIGS.

- 2A-C. The frame has at least four sides, or walls, 60a-d, and may be
10 constructed of a metal, such as aluminum or steel, or another substantially or rigid material such as wood, ceramic, or hard plastic. The bottom frame 10 has a resilient gasket or O ring (not shown) which may be silicone, rubber, or other synthetic material. The O ring is affixed in the channel groove 40 that is recessed in the interior upper surfaces of the frame walls 60a-d. There is also
15 a metal sheet 50a adapted to the bottom surfaces of the frame walls 60a-d; in one embodiment the sheet is made of aluminum. The edges of the frame walls 60a-d and the edges of the bottom metal sheet 50a may have holes (not shown) for receiving fasteners 55, as shown in FIGS. 2B-C, and/or fastening by welding, riveting, or using a metal adhesive to seal the joints of the respective
20 frame walls. Alternatively, these parts may be permanently affixed, for example, the frame walls 60a-d may be constructed from a solid material such that there are no joints between the walls. The holes are aligned with corresponding holes in either the metal sheet 50a or frame walls 60a-d. At least two opposing frame walls 60a and 60c of the bottom frame 10 have at
25 least one flow port 36 that allows flow along the entire width of the frame walls

60a, 60c extending from the outside vertical surfaces 95a, 95c to the inside vertical surfaces 96a, 96c of the frame walls 60a and 60c. Hose barbs 35 are adapted to flow ports 36. At least two opposing frame walls 60a and 60c, 60b and 60d, or both have fasteners 45 which are any type of couplers or other
5 attachments to mate assembly 10 with the top assembly 25, such as with at least one half of a draw latch 45a adapted to their outer vertical surfaces 95a-d.

Center Frame

The center frame 15, or carrier plate, with various components is shown in FIGS. 1A, 3A, 3B, and 3C. As shown in FIGS. 3A and 3C, the frame
10 15 has at least one internal support 65 adapted to inner surfaces 100a and 100b of the frame walls 61b and 61d in the interior of the frame 15, and may have at least one internal frame support 66 adapted to the inner surfaces 105a and 105b of the frame walls 61a and 61c. Along its length, internal support 65 has a plurality of recessed flow vents 85 oriented in the direction of coolant flow
15 to conduct coolant and allow coolant flow through the center frame 15. The walls of the center frame 15 are constructed of any material, and may be jointed or continuous, as previously described for the bottom frame 10.

The center frame 15 has a substantially rigid sheet 50b of a material, such as a metal or ceramic, that is adapted to the top surface (not
20 shown) of the frame walls 61a-d. In one embodiment, sheet 50b has a mirrored-finished metal surface. The mirror-finished surface 51b of sheet 50b may also optionally be coated with a non-adherent material such as TEFLONTM. The top surfaces of the frame walls 61a-d are adapted to a surface of sheet 50b using, for example, a metal adhesive, weld, or rivet.

Top Frame

With reference to FIG. 4A, top frame 25 has four walls 62a-d, at least one support 110 connecting the interior surfaces 115b and 115d of walls 62b and 62d, and may have at least one internal support 111 connecting the 5 interior surfaces 115a and 115c of walls 62a and 62c. The assembly, construction, and composition of walls and supports of the top frame 25 are as previously described for bottom frame 10. Internal support 110 has a plurality of recessed flow vents 90 that extend the entire width of the support 110 and are oriented in the direction of coolant flow. At least two opposing frame walls, 10 for example 62a and 62c, 62b and 62d, or both have at least one flow port 38 that extends the entire width of the frame walls 62a, 62c and hose barbs 37 adapted to the flow ports 38. At least two opposing frame walls 62a and 62c, 62b and 62d, or both have couples, fasteners, or attachments 46 such as a draw latch adapted to outer vertical surfaces 120a-d of the top frame walls 62a- 15 62d. The couplers, fasteners, or attachments 45, 46 are mated to secure the center frame 15 between the top frame 25 and bottom frame 10. Previously described surfaces 51c and 51d are adapted to the top and bottom surfaces of the frame walls 62a-d, both of which have holes (not shown) for receiving 20 fasteners 55 which are aligned with corresponding holes in a frame wall or a sheet, and forms a coolant chamber 128 for receiving coolant 125 as shown in FIG. 1C.

Apparatus Assembly and Matrix Formation

Referring to FIGS. 1A, 1B, and 1C to assemble the apparatus 30 a resilient material (for example, a gasket) or member (for example, an O ring) (not shown) to make a fluid-tight joint, is placed in the channel groove 40 of the bottom frame 10. The center frame 15 is placed on top of the bottom frame 10, with the resilient gasket or O ring forming a seal between center frame 15 and bottom frame 10, thus forming a coolant chamber 129 for receiving coolant 125. A spacer gasket 20 is placed on the top surface 51b of the metal sheet 50b, such that a portion of the spacer gasket 20 is flush with three of the outer edges of the center frame 15. Optionally, an additional sheet 53 of a non-adherent material (i.e., TEFILON™) is overlaid on the spacer gasket 20, with the edges of the sheet 53 extending to the outer edges of the spacer gasket 20. The top frame 25 is then placed over the spacer gasket 20 so that the bottom surface 51c of frame 25 is in contact with either the spacer gasket 20 or the optional sheet 53. The frames 10, 15, 25 are fastened together, for example, by securing half drawn latches 45 and 46 to each other.

As illustrated in FIG. 1C , a chamber 75, to contain a matrix-forming fluid defined by the interior surfaces 22a-c of spacer gasket 20, the bottom optional surface 51c of sheet 50c of top frame 25, or a surface of the optional sheet 53, and the top surface 51b of sheet 50b of center frame 15. The chamber 75 has an opening 76 on one side to introduce the matrix-forming fluid and to insert the spacer gasket plug 21. The inner surface 23 of the spacer gasket plug 21 forms a wall of the chamber 75 when the spacer gasket plug 21 is inserted into the chamber opening 76.

The matrix-forming fluid is introduced into the chamber **75** of the apparatus **30**, either through opening **76** or by another means, substantially filling the entire chamber **75**. In one embodiment, the fluid is introduced incrementally, for example, in increments of about 20 ml-30 ml, with intermittent tapping of the apparatus **30** to avoid trapping bubbles in the fluid. Spacer plug **21** is then inserted into the opening **76** to seal the chamber **75**, thus containing the matrix-forming fluid. The matrix-forming fluid contained within chamber **75** is then cooled at a controlled rate to solidify the matrix-forming fluid and thereby form the matrix.

As illustrated in FIG. 1B, the apparatus **30** is cooled by indirect contact of the chamber **75** with a source of coolant **125**, via a conduit such as a hose **126** or other type of tubing connected to hose barbs **35** and **37**. The conduit **126** may be connected to a coolant circulator **127** for recirculating flow of coolant **125**. Alternatively, the coolant **125** may exhaust. In either case, coolant **125** flows directionally within the walls **60a-d** of the bottom frame **10** and the walls **61a-d** of the center frame **15** which are fastened together, and within the walls **62a-d** of the top frame **25**, for a time and at a flow rate and temperature sufficient to effect controlled freezing of the matrix.

Use of a system that flows or recirculates coolant **125** is advantageous for several reasons. The system is closed, and hence is less hazardous and more contained than an open system, such as a static controlled-temperature bath. The system prevents loss of the coolant **125**, and allows a coolant-containing reservoir of any volume to be used. The system maintains a more stable temperature and, therefore, generates a more stable rate of cooling. Control of the rate of cooling is a factor in the determination of

the distribution of reticulations of the polymer that forms the matrix. A stable controlled rate of cooling results in matrices having a reproducibly consistent and desirable distribution and quality of reticulations.

While the chamber **75** of apparatus **30** has six surfaces which are
5 identified by numerals **22a-c**, **23**, **51b**, and **51c** (the boundaries of the "box") ,
four of these surfaces, defined by numerals **22a-c** and **23**, are insulated by the
gaskets **20**, **21** between the surfaces of sheets **50b** and **50c**. Therefore,
virtually all of the heat is removed, and the cooling is regulated, through these
10 two surfaces of sheets **50b** and **50c**. In this respect, the cooling of the matrix-
forming fluid could be referred to as occurring between the two surfaces **51b**
and **51c**.

Any type of fluid coolant **125** that is able to absorb heat and to
lower the temperature in the chamber **75** sufficient to freeze the matrix-
containing fluid at a controlled rate, that is, to effect a decrease in temperature
15 from about 4°C to about -70°C in less than about two hours, for example, from
about ten minutes to about twenty minutes, or less than about one-half hour.
Fluid cooling advantageously occurs with efficiency and predictability, and
furthermore is more easily regulated than air cooling, in the same manner as
fluid cooling of an automobile engine occurs more efficiently than does air
20 cooling. Examples of coolants which may be used include, but are not limited
to, alcohols (for example, ethanol, propanol, etc.), polyethylene glycol, oils,
silicone oils, or any fluid with low viscosity at temperatures down to -70°C, etc.
In one embodiment, the temperature of the coolant is between about 0°C and
about -200°C. In another embodiment, the temperature of the coolant is
25 between about -20°C and -70°C. In yet another embodiment, the temperature

of the coolant is between about -40°C and -70°C. These temperatures regulate the average size and distribution of the reticulations in the matrix. As one example, the matrix-forming fluid is exposed to 95% ethanol that has been chilled to -45°C for between 60 minutes and 120 minutes. Other embodiments
5 are also within the knowledge of one skilled in the art.

Gases that have a vapor transition temperature which permit freezing of the matrix-forming solution can also be used as a coolant, as known to one skilled in the art. For example, gaseous nitrogen (vapor transition temperature -196°C), known to one skilled in the art to effect controlled rate
10 freezing of cells in culture, is sufficiently cold to freeze the matrix-forming fluid. In embodiments using a gas coolant, the flow of gas may be increased to compensate for the decreased efficiency of cooling with a gas, compared to a liquid which can absorb more heat. Increased flow of a gas may be easier to achieve than with a liquid because a pump would not be required. Other
15 advantages of using a gaseous coolant are that small leaks would not be problematic, as they may be with liquid cooling, and a gas coolant system would be cleaner because there would be no attendant liquid spills.

For static cooling, the embodiment of the apparatus 30a illustrated in FIGS. 1D and 1E is used. The chamber 75 is configured without
20 the bottom frame 10 and without the sheet 50d of the top frame 25. The chamber 75 is sealed at the perimeter 22a-c and 23 to prevent contact between the matrix-forming solution and the liquid coolant, because contact would result in failure to freeze or melting of the matrix. Alternatively, the chamber 75 within apparatus 30a is sealed at only three of the four sides (for example, 22a-c),

leaving the fourth side unsealed. The apparatus **30a** is then immersed or dipped into a bath of fluid coolant up to, but not over, the unsealed side.

After exposing the matrix-forming solution to a coolant under conditions sufficient to freeze the solution and form a matrix, the closures **45** 5 are released. The top frame **25** is carefully removed from the center frame **15**, upon which the matrix is supported on the surface **51b** of sheet **50b**. The non-stick sheet **53**, if present, is removed carefully from the top surface of the frozen matrix. The spacer gasket **20** and spacer gasket plug **21** may optionally 10 be removed from the center frame **15** and the matrix. The center frame **15** is removed from the bottom frame **10**. The center frame **15**, supporting the matrix 130 on surface **51b** of sheet **50b**, is placed in a freeze-drying device to lyophilize the ice from the matrix **130**.

The rate of freezing of the matrix-forming fluid may be controlled by adjustment of one or more variables that contribute to the rapid heat transfer 15 from the matrix-forming solution to the ambient environment. Therefore, the invention includes use of an apparatus **30** or **30a**, to contain and contact the matrix-forming fluid, made of a material which allows highly efficient heat transfer. The apparatus **30** itself allows regulation of several variables during the freezing process. These variables include the shape and dimensions of the 20 apparatus **30**, the volume of matrix-forming fluid that is contained, and the thermal conductivity and specific heat of the materials which contact the matrix-forming fluid, that is, the specific material forming the substantially rigid and flat sheets **50b** and **50c** which contact the matrix-forming fluid. One example of such a material is metal (for example, aluminum, stainless steel, etc) which is a 25 good conductor of heat. Desirably, the material is also substantially rigid to

resist deformation, and contains a nonstick and/or polished surface that is in contact with the matrix-forming fluid.

The size and uniformity of the reticulations in the matrix formed from the matrix-forming fluid result from the size of ice crystals formed during the freezing process, because the ice displaces the solute in the fluid and thus determines the distance between reticulations. The size of ice crystals is inversely proportional to the rate of freezing. The rate of freezing is inversely proportional to the temperature of the coolant, and directly proportional to the thermal conductivity and specific heat of any materials which contact the matrix-forming fluid during the freezing process.

The size and distribution of the reticulations in the matrix may also be regulated by the temperature at which the components in the matrix-forming fluid freeze. The distance between the reticulations is inversely proportional to the concentration of components and to the rate of freezing of the matrix-forming fluid, and directly proportional to the concentration of water in the matrix-forming fluid. The thickness of the matrix is directly proportional to the concentration of components in the matrix-forming fluid. The concentrations of components in the matrix-forming fluid may be formulated to yield a combined % wt/vol concentration which decreases the average distance between the reticulations, and increases the thickness of the matrix. In one embodiment, combined component concentrations in the range of about 0.10%^{wt/vol} to about 2.0%^{wt/vol} matrix compounds may be used. In another embodiment, combined component concentrations in the range of about 0.50%^{wt/vol} to about 1.80%^{wt/vol} may be used. The thickness of the matrix is also is directly proportional to the thickness of the spacer gasket **20** and spacer gasket plug **21** used in the

chamber 75. In one embodiment, the chamber 75 is configured to result in a matrix having a thickness is in the range of about 0.1 mm to about 3.0 mm.

The responses to the freezing temperature of the size and distribution of the reticulations prepared using the inventive method and 5 apparatus may be determined by measurement of scanning electron micrographs (SEM) of the rehydrated and dried matrix. In one embodiment, the reticulations are distributed in the shape of ellipsoid spheres and have an average diameter in the range of about 50 μm to about 150 μm .

An apparatus 30 for preparing a biocompatible matrix, and a 10 method of using the apparatus 30 to prepare a biocompatible matrix, are thus disclosed. The inventive apparatus 30 and method provide a matrix capable of supporting cells to form an implantable or engraftable surgical device, such as a cultured skin device for temporary or permanent skin replacement on a burn or other wound. The matrix is used to support one or more layers of cultured 15 dermal cells, which serve as a lamination layer for epidermal cells. Other variations or embodiments of the invention will also be apparent to one of ordinary skill in the art from the above description. Thus, the forgoing embodiments are not to be construed as limiting the scope of this invention.

What is claimed is: